A COMPARATIVE STUDY TO EVALUATE THE ADHERENCE POTENTIAL OF CANDIDA ALBICANS ON CONVENTIONAL HEAT CURE POLYMETHYL METHACRYLATE RESIN AND FLEXIBLE DENTURE BASE RESIN

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BRANCH I

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CERTIFICATE

This is to certify that the dissertation titled "**A Comparative Study to Evaluate the Adherence Potential of Candida Albicans on Conventional Heat Cure Polymethylmethacrylate and Flexible Denture Base Material**" is a bonafide record of work done by **DR. S.GEETHA** under my guidance during her postgraduate study period 2009 – 2012.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY**, in partial fulfilment for the degree of **Master of Dental Surgery** in **Prosthodontics and Crown and Bridge.** It has not been submitted (partially or fully) for the award of any other degree or diploma.

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1. Introduction

INTRODUCTION

Candida albicans and related Candida species are the most common opportunistic pathogens and these organisms are widely known to cause oral candidiasis and denture stomatitis. The conversion from commensalism to parasitism is usually associated with intra-oral environmental changes (e.g. unhygienic prostheses, Xerostomia) and/or systemic factors such as diabetes and immunodeficiency[19]. Denture stomatitis is an inflammatory condition of the palatal mucosa characterized by pinpoint hyperemia, diffuse hyperemia or inflammatory hyperplasia. Candida associated denture stomatitis affects up to 67% of the denture wearers. The other etiological factor which favors denture related stomatitis includes reduced vertical dimension, unstable occlusion and continuous wearing of prosthesis [10].

The colonization of candida species on the tissue surface of denture depends on several factors, such as adherence potential of yeast cells, interaction with oral commensal bacteria, redox potential of the site and surface properties of the acrylic resin[28]. The pathogenicity of the plaque can be enhanced by the factors stimulating yeast propagation, such as poor oral hygiene, high carbohydrate intake, reduced salivary flow and continuous denture wearing.

Additional factors that can modulate host-parasite relationship and increase the susceptibility to Candida associated denture stomatitis may be aging, malnutrition, immune suppression, radiation therapy, diabetes mellitus and possibly treatment with broadspectrum antibiotics[44].

Samaranayake L P and McFarlane F W[27] study reported a positive correlation between yeast concentration and number of cells adherent on acrylic surfaces. Evidence supports that unclean dentures and poor hygiene care are the major predisposing factors in denture stomatitis. The tissue surface of dentures usually shows micro pits and micro porosities that harbor microorganisms which are sometimes beyond the reach of mechanical or chemical cleaning methods.

Heat cure Polymethyl methacrylate acrylic resins are conventionally used to construct dentures. It was introduced in 1931 by **Dr. Walter Wright**[3]. The properties that have contributed to the success of this material as a denture base are its ease in availability, handling, processing, cost effectiveness, repairability, low solubility, better fit and stability in oral environment[1]. The properties of the material are acceptable but not ideal in all aspects. Despite its widespread popularity, its rigidity limits, its use in conditions like unyielding undercuts, pronounced tuberosities, tori

and bulging alveolar ridges[37]. Polymethyl methacrylate has some inherent weakness like its susceptibility to fracture due to its low impact strength, low resistance to flexural fatigue, poor resistance to abrasion and allergic reactions[17].

Numerous approaches to enhance the physical properties of Polymethyl methacrylate have been attempted[4,15,31,34]. These include chemical modification & reinforcement of Polymethyl methacrylate with other materials and further to the development of an alternative material such as polyamides, epoxy resin, polystyrene, vinyl esters, polycarbonate and nylon etc. Nylon is a generic name used for certain types of thermoplastic polymers belonging to the class known as polyamides. Nylon polyamides were introduced by **W.H.Carothers** and associates of the Du Pont Chemical Co, America (1928 – 1938)[37]. It was first used in the construction of denture bases in 1950s. Nylon 66 and Nylon 610 were the principal materials used as denture base. As compared to Polymethyl methacrylate this material was less rigid, highly resilient and resistant to abrasion and unbreakable[22]. These properties are very helpful to fabricate maxillofacial obturators[39] and partial dentures[38].

The efficacy of flexible resin over PMMA was its ease of insertion and handling, flexibility, excellent surface finish, reduced bulk, better tissue adaptation and retention[39]. Certain disadvantages

of early form of nylon polyamides were difficulty in processing, tendency to deteriorate, color instability, high water absorption and surface roughness after few weeks of wear[13]. Advantages of these materials included its monomer free composition and elimination of metal clasps[36] for retention. Valplast is a nylon superpolyamide[37] (Valplast international corp., New York, USA) and was developed by **Arpad F Nagy** and **Tibor F Nagy** in 1950's as denture base material[43]. So its use was restricted to limited conditions like repeated denture fractures, proven allergy[36] to Polymethyl methacrylate, lack of neuromuscular coordination and construction of orthodontic appliances.

According to several in vitro studies, the microbial contamination of denture acrylic resins occurs very quickly, and yeast seems to adhere well to denture base materials. Although denture stomatitis and angular chelitis do not reflect a serious predisposing disease or abnormality, it can lead to severe infections in the immunocompromised host. Simultaneously, most of the patients are aged and medically compromised and many of them are affected by diabetes, post radiation hazards, and Xerostomia etc. In these conditions salivary flow is affected and favoring the growth of oral microorganism. The denture base acts as reservoir for colonization for organisms.

Kamran[20] proved that diabetic patients are prone to candidal colonization. The improvised nylon flexible denture base materials are available in market and their usages have increased in recent years. Very few studies have been conducted on these materials on its surface roughness, contact angle, biofilm formation and candidal adherence compared to Conventional heat cure resins.

The aim of this study is to evaluate and compare the adherence potential of candida between the flexible denture base resins (Valplast) and conventional Polymethyl methacrylate resins (DPI).

2. Aims and Objectives

AIMS AND OBJECTIVES

AIM:

The aim of the study is to compare the adherence potential of Candida albicans between flexible denture base resin (Valplast) and conventional heat cure Polymethyl methacrylate resin (DPI).

OBJECTIVE:

- To evaluate the adherence potential of candida albicans on flexible denture base resin (VALPLAST) and conventional polymethyl methacrylate acrylic resin(DPI)
- To further evaluate the adherence potential of Candida albicans on these two different types of acrylic resin in cleaned and uncleaned state.

3. Review of Literature

REVIEW OF LITERATURE

Stern M N[37] in 1964 described Valplast material as super polyamide which had flexibility in thin sections (2mm) and rigid in thick sections (10mm).He recommended Valplast for obturator in cleft palate patients, gingival veneers to mask wide Interdental spaces.

Barry Shipman[2] in 1979 did a study to isolate, identify, and determine the clinical significance of Candida species present in the saliva of patients receiving immunosuppressive anticancer chemo therapy. The aim of the study was to determine the effect of anticancer chemotherapeutic drugs on the salivary pH, and to determine the correlation between salivary pH, the species of Candida, the incidence of Candida, and the specific drug regimen. The administration of six drugs, 5FU, Methotrexate, Vincrestine, Cytoxan, Methyl CCNU, and Adriamycin caused a significant decrease in 50% of the salivary pH fall below 6.6. This pH change may create an environment in which Candida species will grow more easily.

Ejvind Bud and J Brgensen[6] in 1979 reviewed materials and method for cleaning dentures and discussed different means of keeping dentures plaque-free. Products containing insoluble calcium

carbonate are highly abrasive, whereas dentifrices containing soluble sodium bicarbonate are less abrasive. Specially developed denture-cleansing paste containing zirconium has been found to be superior to a number of commercially available pastes for cleaning and polishing dentures and decreasing acrylic resin abrasion. Ultrasonic treatment of dentures in disinfectant solutions increases the disinfectant's effectiveness and does not deteriorate the polished denture surface.

Mc courtie J, Julia Douglas[26] in 1981 determined the adherence of C. albicans to acrylic after growth in defined medium containing glucose, sucrose, galactose, fructose, or maltose as the carbon source. Adherence of C. albicans to acrylic was inhibited when saliva-treated acrylic strips were used in assays or when the yeasts were suspended in saliva; the attachment of certain oral bacteria to hydroxyl apatite is similarly inhibited by saliva. The environmentally induced changes in yeast cell surface composition likely to influence adherence in vivo as well as in vitro, whereas dietary sucrose probably enhances the adherence of C.albicans to acrylic dentures. Other sugars may have a similar effect. In this connection, diabetics and patients undergoing antibiotic or steroid therapy have elevated levels of salivary glucose situations are known to predispose individuals to candidiosis.

Tamamoto et al[38] in 1985 evaluated the ability of enzymes to remove C.albicans from an acrylic resin surface. They have tested acrylic resin samples with yeast lytic and proteo lytic enzymes. They have found out that yeast lytic enzyme destroy cell walls. Proteolytic enzymes, with the exception of trypsin, could also remove C.albicans. These results suggest that c.albicans adhered to the acrylic resin surfaces by protein. They suggest that these compounds are potentially useful denture cleansers.

Klotz et al[21] 1985 in their study they have found out that the forces responsible for candidal adherence are attractive London-van der Waals forces (or hydrophobic forces) and electrostatic forces. The hydrophobic affinity of yeasts was determined by (i) a water-hydrocarbon two-phase assay and by measurement of the contact angle (0) of a liquid droplet on a monolayer of yeast cells. The adherence of yeasts to polymers of increasing hydrophobicity (determined by the contact angle method) was directly proportional to contact angle. Yeast surface charges were altered by selectively blocking amino and carboxyl groups. The more positively charged yeasts adhered in greater numbers. Increasing the molarity of NaCl increased yeast adherence. These forces probably contribute to the negative cooperativity (determined by Scatchard and Hill plot) that characterizes the adherence of yeasts to polymers.

Minagi et al[28] in 1985 evaluated the effects of hydrophobicities of substrate surfaces on microbial adherence by using Candida albicans, Candida tropicalis and 21 denture base resin materials. With increasing surface free energy of resin plates, increasing adherence of C. albicans and decreasing adherence of C. tropicalis were observed. The surface free energy of C. albicans is higher than that of all resin material surfaces, and C. tropicalis has surface free energy lower than that of all materials used. In calculation of the changes of free energy accompanying the adherence, the higher adherence tendency was accompanied by a lower value for the free energy change in both species. From a different standpoint, the closer the surface free energy of the substrate surface and the microorganism, the higher was the probability of adherence.

Mac courtie et al[25] in 1985 did a study to evaluate the effect of chlorhexidine gluconate on the adherence of candida species to acrylic resin. They have found that adherence was significantly reduced by pretreatment with chlorhexidine; maximal inhibition was achieved by incubation at room temperature for 30 min in 2% chlorhexidine.

Yeasts grown in high concentrations of galactose, which were the most adherent to acrylic, were also the most sensitive to the fungicidal action of chlorhexidine gluconate, whereas those

grown in a low concentration of glucose were the least adherent and also the most resistant.

Mc courtie et al[27] in 1986 have done a study to evaluate the effect of saliva and serum on the adherence of five strains of *Candida albicans* and one each of C. *tropicalis* and *C. glabrata* to **chlorhexidine-pretreated** acrylic. Results have revealed that four-fold dilution of saliva or serum significantly inactivated the fungicidal effect of chlorhexidine gluconate. Pretreatment of the acrylic with unstimulated mixed saliva for 30 min led to a reduced adherence for all the *Candida* strains tested, whilst a similar pretreatment with serum slightly increased adhesion. Moreover treatment of saliva- or serum-coated acrylic with chlorhexidine gluconate **2%** reduced adherence by between 19% and **86%.** The inhibition of yeast adherence by chlorhexidine persisted for up to 19 days after the exposure of the acrylic strips to the disinfectant.

Dills et al[5] in 1988 evaluated the ability of various denture cleanser products. These products included an abrasive denture paste and an alkaline peroxide-based chemical soak product used according to the manufacturer's directions. These products included an abrasive denture paste and alkaline peroxide-based chemical soak products. The results showed soaking with the denture cleanser caused a significantly greater reduction of microorganisms than did

brushing with the denture paste. Further, combining brushing with the soak did not reduce the level of recoverable microorganisms significantly more than soaking alone. These results support the need for use of a denture cleanser in addition to brushing with a denture paste for proper denture hygiene.

Verran et al[40] 1997 compared the retention of Candida albicans on smooth and rough acrylic resin and silicone surfaces after a washing procedure to determine the effect of surface roughness on prosthesis infection and hygiene. An increase in surface roughness facilitated yeast retention on silicone and acrylic resin surfaces. The intaglio of the maxillary denture was prone to colonization by C. albicans and was not smooth. Surfaces that were as smooth as possible were more desirable in terms of cleanability and infection.

Waltimo et al[43] in 2001 done a study to determine the adherence of yeasts to newly polymerized and water stored denture base polymers using four Candida strains with different cell surface hydrophobicities. They found out that yeast cells seem to adhere less to newly polymerized than water stored denture base polymer. They may be due to the release of residual monomer from the newly polymerized material.

Ye Jin et al[44] in 2004 examined the effect of human whole saliva and dietary sugars, glucose and galactose on the adhesion and biofilm formation of Candida albicans. Biofilms of C. albicans isolate were developed on polystyrene, flat-bottomed 96-well microtiter plates and monitored using ATP bioluminescence and tetrazolium. Compared with a glucose supplemented (100mm) medium, galactose containing (500mM) medium generated consistently lower levels of both candidal adhesion and bio-film formation (all $P < 0.05$), but a higher pace of biofilm developed over a time (96 h). With the presence of an immobilized saliva coating had little effect on either the candidal adhesion or biofilm formation, the addition of saliva to the ffected biofilm formation especially on day 3 and 4, without any significant effect on dhesion. Biofilm formation of C. albicans within the oral milieu appears to be modulated to varying extents by dietary and salivary factors.

Leonard Garth Lowe[22] in 2004 used flexible material in patients with buccal undercut in the maxillary tuberosity. He described a technique for incorporating flexible denture flange to an acrylic denture, which gave more comfort to the patient during removal and insertion of the prosthesis. It gave excellent border seal which enhanced retention. He also stated that this material showed minor color instability and microbial colonization.

Granger C S[14] in 2005 conducted a study to evaluate the relationship of candidal adherence to surface energy. Surface energy of resins was calculated by the contact angle method. According to this study, variations in surface energy that result from differences in the composition of the different PMMA resins appear to have no influence on the adhesion of C. albicans, and denture stomatitis.

Moura J S[29] in 2006 evaluated the influence of polymerization methods of acrylic resins and human whole saliva on the adherence of Candida species to acrylic resin surfaces. Result suggested that saliva was capable of reducing the adherence of Candida species, whereas roughness and free energy did not influence the adherence rates. As growth on surfaces is a natural part of the Candida lifestyle, its colonization in denture users may be expected. The presence of human whole saliva, however, decreased the overall yeast adherence to the acrylic resin surface, whereas surface roughness and free energy did not interfere with the adherence of Candida species.

Gunjan dhir et al[15] in 2007 evaluated the physical properties of a modified denture base resin for denture fabrication. Specimens made from heat polymerizing resin Lucitone 199 were used as the control group. The two experimental groups, E-10 and E-20, had 10% and 20% monomer substituted with an experimental

phosphate-containing monomer. Flexural strength and modulus, water sorption, solubility, and color stability tests were conducted to ensure compliance with ADA specification No. 12. Water diffusion coefficient into the resins and stain ability were also assessed. Results have revealed that there was an overall decline in all properties with the addition of the experimental phosphate compound.

The flexural strength and flexural modulus, water absorption and solubility however, were within the ADA specifications. The diffusion coefficients were significantly different for the three groups. Staining and color specimens showed no significant difference ($p > 0.05$) among the three groups. Within the limitations of this study, the physical properties of the phosphate denture base resin at 10% should be suitable for denture fabrication based on the properties assessed.

Emami et al[7] in 2007 conducted a study to verify the relationship between the presence of denture stomatitis and the frequency of myceliated colonies of C albicans isolates in denture wearers. The results were prevalence of denture stomatitis was 77.5%. The ability of C.albicans strains isolated from dentures to produce myceliated colonies may not be directly involved in denture stomatitis.

Fernandes et al[8] in 2007 have done a Study to compare the efficacy of three denture brushes (Bitufo-B; Medic Denture-MD; Colgate-C) on biofilm removal from upper and lower dentures using a specific dentifrice (Corega Brite). The correlation between biofilm levels on the internal and external surfaces of the upper and lower dentures was also evaluated. A microbiological assay was performed to assess the growth of colony-formed units (CFU) of Candida yeasts on denture surface. All denture brushes evaluated in this study were effective in the removal of biofilm. There was better correlation of biofilm levels between the surfaces for the lower dentures, and between the dentures for the external surface. There was no significant difference among the brushes regarding the frequency of yeasts.

Cervantes et al[3] in 2009, evaluated the effect of 5% sodium bicarbonate on the adherence of *Candida albicans* to thermally activated acrylic resin. They have found out although 0.12% digluconate chlorhexidine was more effective in the reduction of *Candida albicans* adherence values to thermally activated acrylic resin, 5% sodium bicarbonate also proved to be a viable alternative.

Paranhos et al[30] in 2009 conducted a study to evaluate the effect of three denture hygiene methods against different microbial biofilms formed on acrylic resin specimens. Acrylic resin specimens

were contaminated by microbial inoculums with 10^{6} colony forming units. After inoculation specimens were incubated for 48 hours at 37^{0} C then they were cleansed by the following methods;(1) Chemical: immersion in an alkaline peroxide solution for 5 minutes; (2) mechanical: brushing with a dentifrice for removable prostheses for 20 seconds; (3) a combination of chemical and mechanical methods .Results showed that chemical , mechanical, and combination methods showed no significant difference in the reduction of CFU for S.aureus, S.mutans(ATCC and field strain), and P.aeruginosa. Mechanical and combination methods were similar and more effective than the chemical method for E.faecalis, C.albicans(ATCC and field strain), and C.glabrata. The combination method was better than the chemical method for E.Coli and C.tropicalis, and the mechanical method showed intermediate results.

Kamran et al[20] in 2009 done a study to evaluate and compare *Candida* colonization in denture of diabetic patients and non-diabetic control group. Results have revealed that diabetes mellitus can increase colonization of *Candida* in denture and mouth. By elimination of local and systemic factors in diabetic patients and improving their oral health care, *Candida* colonization and the risk of oral and systemic candidiasis will be decreased.

Park et al[31] in 2009 conducted a study to examine the mechanical properties of a new surface-modified denture resin for its suitability as denture base material. Correlation existed between the physical properties and the anti-fungal activity of surface-charged resins. In the present study, the greatest decrease in transverse and flexural strengths occurred when the ratio of methacrylic acid content was increased from 5% to 10% PMMA (P <.05). Interestingly, it was also between these two groups that the most significant reduction in adhesion of *C. albicans* occurred. As the ratio of methacrylic acid content was increased, the adhesion of *C. albicans to* resin surfaces decreased; however, the physical properties declined in consequence.

Hashiguchi M[16] 2009 in their study evaluated the bactericidal efficacy of 1.00–4.50% glycine-type amphoteric surfactant (Gly) by measuring its microorganism removal rate in denture plaque. Physical and mechanical properties such as surface roughness, color difference, and bending strength of two different denture base resins were determined before and after cleaning in Gly solutions, a commercial denture cleaner, and tap water. The microorganism removal rates of all the Gly solutions were higher than those of a commercial enzymatic denture cleaner (Polident) (p>0.05). The removal rate of *Candida spp*. by Polident was not

significantly different from the removal rate using water. Changes in the surface roughness and color difference among the specimens were slight. There were no significant differences in the bending strengths of the two resins for all concentrations of Gly solution. These results suggested that glycine-type amphoteric surfactant solution may be effective as a denture cleaner in conjunction with an ultrasonic cleaning device.

Gasparaoto et al[11] in 2009 done a study to verify through PCR the presence of C. dubliniensis in palate and maxillary denture samples from 112 denture wearers presenting with or without denture-related stomatitis (DRS). C. dubliniensis was isolated at low rates from both palate (5.3% and 10.7 %) and maxillary denture (5.3% and 8.9 %) samples from wearers regardless of the presence of the disease. However, when C. dubliniensis was detected in individuals with DRS, it was always associated with C. albicans. In addition, results showed that C. albicans was the most commonly identified candidal species in maxillary denture and hard palate samples from DRS patients (78.5% and 89.2 %, respectively) as well as from controls(31.2% and 28.5 %, respectively).

C. dubliniensis was detected in the oral environment of denture wearers. The association of C. dubliniensis with C. albicans occurred in approximately 10% of the DRS cases.

Gasparoto et al[12] in 2009 have done a study to determine ageing-related changes in salivary and blood neutrophils and their potential implications in *Candida*-related denture stomatitis. Results showed a lower number of neutrophils in the saliva from patients presenting *Candida*-related denture stomatitis in comparison to their matched controls. Fewer neutrophils were isolated from the saliva of aged control individuals in comparison to matched younger subject. Systemic neutrophils from elderly showed decreased phagocytosis when compared to the younger ones, regardless of the occurrence of infection. The data suggests that the *Candida* related-denture stomatitis is associated to neutrophils function deficiency, and ageing drastically appears to alter important characteristics of such cells, facilitating the establishment of this infection.

Vieira et al[41] in 2010 evaluated the long-term efficacy of denture cleanser against *Candida* spp. biofilm recolonization on liner surface. Specimens were fabricated of a poly (methyl methacrylate)-based denture liner and had their surface roughness evaluated at baseline and after cleansing treatments. *C. albicans* or *C. glabrata* biofilms were formed on liner surface for 48 h, and then the specimens were randomly assigned to one of cleaning treatments: two alkaline peroxides (soaking for 3 or 15 min), 0.5% sodium hypochlorite (10 min) or distilled water (control; 15 min).

After the treatments, the specimens were sonicated to disrupt the biofilm, and residual cells were counted (cell/mL). Long-term effectiveness of the cleaning process was determined by submitting a set of cleaned specimens to biofilm growth conditions for 48 h followed by estimation of cell counts. No significant difference ($p > 0.05$) was observed among the *Candida* species regarding the recolonization condition. Alkaline denture cleansers showed similar cleaning performance and both differed from the control ($p < 0.001$). Sodium hypochlorite was the only treatment that removed biofilm efficiently, since no viable cells were found after its use. They concluded that the alkaline peroxide denture cleansers are not effective in removing *Candida* spp. biofilm from denture liner surfaces and preventing biofilm recolonization.

Vural C[42] in 2010 investigated the *C. albicans* adhesion to cold- and heat-polymerized soft lining materials. They were initially incubated in two different artificial body fluids, namely saliva and nasal secretion, and examined the surface roughness the materials (cold and heat polymerized soft liner) tested *in vitro*. Cold-polymerized soft-lining material tested showed more *C. albicans* adherence in both saliva and nasal secretion being widely spread onto the material than that of the heat-polymerized one, where *C. albicans* accumulation was observed more concentrated in

blastospore morphology. The surface roughness of cold polymerized soft liner (Visco Gel) was significantly higher than heat-polymerized soft liner (Molloplast B). Cold-polymerized soft liner (Visco Gel) with roughened surface showed a greater adherence of *C. albicans*. Contamination of the soft liners in artificial nasal secretion led to decreased *C. albicans* adherence than that of artificial saliva.

Peracini et al[32] in 2010 did a study to measure the color change, surface roughness and flexural strength of heat-polymerized acrylic resin after its immersion in denture cleansers, simulating a 180 day use. They found out that the denture cleansers produce color changes, increased surface roughness and diminished the flexural strength of the acrylic resin.

Jeniel Nett et al[19] in 2010 developed a rodent acrylic denture model and characterized the *Candida albicans* and mixed oral bacterial biofilm formation, architecture, and drug resistance *in vivo*, using time course quantitative culture experiments, Confocal microscopy, scanning electron microscopy, and antifungal susceptibility assays. They also examined the utility of the model for measurement of *C. albicans* gene expression and tested the impact of as specific gene product (Bcr1p) on biofilm formation. Finally, he assessed the mucosal host response to the denture

biofilm and found the mucosal histopathology to be consistent with that of acute human denture stomatitis, demonstrating fungal invasion and neutrophil infiltration.

Chladek et al[4] in 2011 done a study to avoid candidal colonization in denture soft lining materials; they modified soft liner materials with silver nanoparticles (AgNPs). The modification process was conducted by dissolving both material components (base and catalyst) in a colloidal solution of AgNPs and evaporating the solvent. The AFE of the achieved composites containing 10 to 200 ppm AgPNs was 16.3% to 52.5%. This level of AFE should be capable of preventing colonization of *Candida albicans* on soft denture lining.

Ryalat et al[34] in 2011 evaluated the release of chlorhexidine as an antifungal drug from doped self-cured poly methyl methacrylate (PMMA) acrylic resin and the effect of the drug released on the growth of *Candida albicans.* In their in vitro study they mixed 10% w/w chlorhexidine powder with polymer (PMMA). Polymer monomer ratio was 5g/3ml. Release of chlorhexidine was evaluated using liquid chromatography, and the effect of the drug on the growth of *C. albicans* was investigated microbiologically using a well" technique on Saboraud culture medium inoculated with a resistant strain of *C. albicans.* They have found out

Chlorhexidine leached steadily out of the acrylic resin into distilled water at mouth temperature, and the sustained drug release continued throughout the 28-day test period. The drug released also demonstrated antifungal activity against the resistant strain of *C. albicans.*

Rajani et al[33] in 2011 conducted a study to evaluate the surface adherence of Candida albicans on different Polymethyl methacrylate resins. They prepared four types of polymethyl methacrylate resin samples. (Self-cure, light cure, Trevalon clear, DPI Heat cure, Lucitone heat cure).The samples were immersed in candidal solution. Results showed adhesion was low in Lucitone199 heat cure denture base material, followed by DPI heat cure denture base resin, which was followed by Trevalon Clear heat cure denture base resin. Lucitone 199(reinforced) had the least adherence of C. albicans.

Shamnur[36] in 2011 reviewed various flexible denture base materials. They said there are wide ranges of flexible materials in the market. They Complete biocompatibility is achieved because the material is free of monomer and metal, these being the principle causes of allergic reactions in conventional denture material (virtually Invisible, Unbreakable, Metal-Free, Lightweight) and incredibly Comfortable. They also advocated brushing a Valplast

appliance is not recommended as this may remove the polish and roughen the surface over time.

4. Materials and Methods

MATERIALS AND METHODS

An in vitro study was done to compare the adherence potential of Candida albicans on heat cure Polymethyl methacrylate resin (DPI) and flexible denture base resin (Valplast) in the Department of Prosthodontics with the assistance from Department of Microbiology.

The materials and methods used for this study have been described as,

1. MATERIALS AND ARMAMENTARIUM

2. METHODOLOGY

a. Preparation of samples.

b. Testing of samples.

1. MATERIALS AND ARMAMENTARIUM

MATERIALS USED	MANUFACTURER
Flexible denture base material	VALPLAST (cartridge system) (pink) Valplast International Corp, New York, USA
Heat cure conventional Polymethyl methacrylate	DPI (DENTSPLY, The Bombay Burmah trading Corporation Ltd. Mumbai)
Saliva substitute	(Wet mouth-ICPA)
Modeling wax	Hindusthan dental products, Hyderabad, India
Sprue wax	Renfert (Germany)
Phosphate buffer solution (PBS)	
80% Ethanol	
Crystal violet	
Candida albicans culture	
Saboraud's medium	
Measuring pipettes, Test tubes	
0.5Mc Farland solution	
Optical microscope	Leica (Germany)

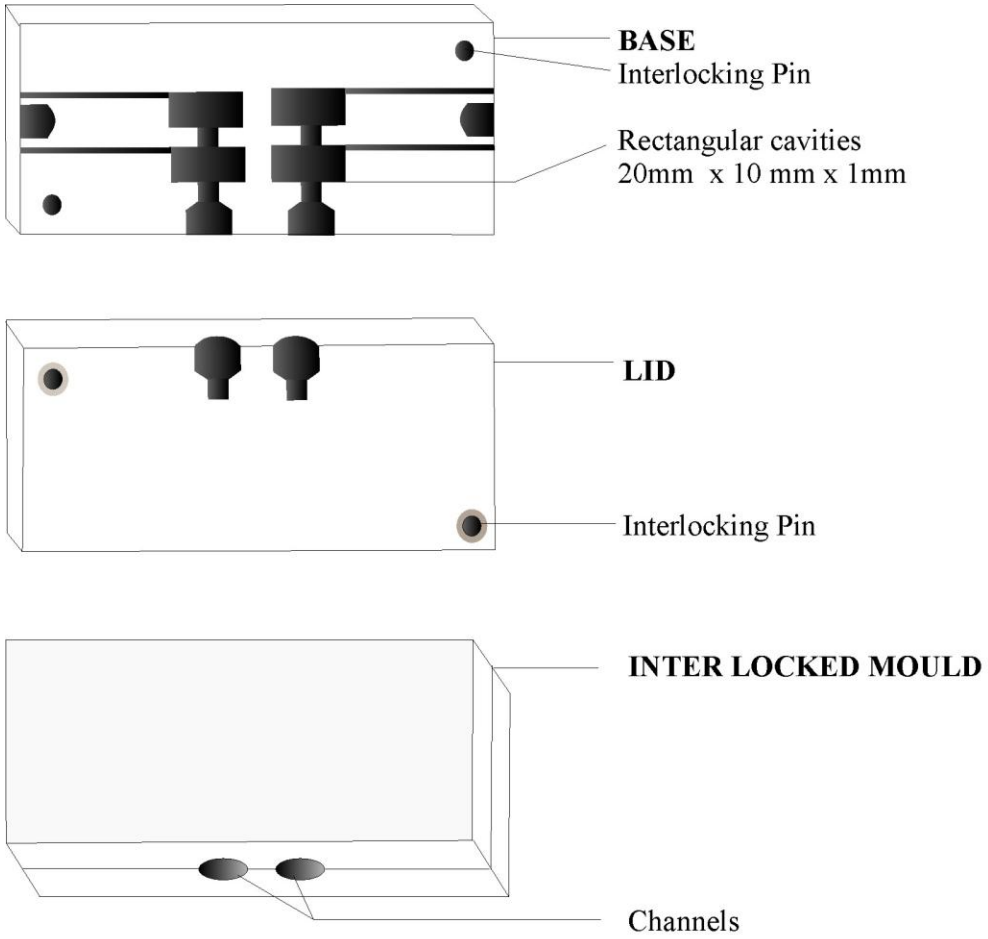
2. METHODOLOGY

a. PREPARATION OF SAMPLES

PREPARATION OF THE WAX PATTERN

A metal mould was fabricated for the purpose of making wax patterns as shown in the schematic diagram below. It has two parts; a) Base, b) Lid. Base has 4 rectangular cavities with dimensions of 20 mm (length), 10 mm (width), and 1mm (depth). Lid is flat with two interlocking slots to seat accurately on the base.

On locking, a channel is formed at the junction of the base and lid through which molten wax can be poured, to make the wax patterns. Petrolatum jelly is applied on the slots for the ease of separation. Wax patterns thus prepared were used in fabrication of valplast and conventional PMMA specimens with the above dimension.



SCHEMATIC DIAGRAM OF THE MOULD

PREPARATION OF VALPLAST SAMPLES–USING INJECTION MOULDING TECHNIQUE: The samples were processed by injection moulding technique by using a flask specially designed by the manufacturer. Wax sprues were attached and the wax patterns invested in the lower part of the dental flask using dental stone (Type 3 gypsum). After the stone had set separating medium was applied and space maintainer for the

cartridge was placed. The counter part of the flask was placed on the base part and the dental stone was poured. After the stone was set, the flask was placed in boiling water for 4-6 minutes. The space maintainer and the patterns were removed from the investment material and dewaxed thoroughly. Separating medium was applied and flask allowed to cool to the room temperature.

The valplast cartridge was placed in the furnace and preheated to a temperature of 287.70 ºC (550º F) for 11 minutes. The stone moulds were exposed under heat lamps and was uniformly heated for 15 to 20 minutes to a temperature of 80°C to avoid any premature freezing of the molten nylon as it entered the mould cavity under pressure. The metal injector was placed in position and along with the cartridge containing melted Valplast; they were placed on to the injection unit. The molten Valplast was then forced into flask using a plunger, the injection moulding pressure being maintained at5 bars for 3 min and then the assembly was removed and disengaged. The flask was bench-cooled for 20 min and then deflasked. The cured valplast samples were removed from the moulds and the sprues were removed with a Valplast specific cutting disc. The surfaces of the specimens were polished using Valplast specific polishing compounds according to the manufacturer's instructions.

PREPARATION OF THE CONVENTIONAL HEAT CURED POLYMETHYL METHACRYLATE SAMPLES

Wax patterns were made using the same metal mould for the fabrication of conventional acrylic samples. The patterns were flasked in Type III dental stone within a metal dental flask. After the gypsum had set completely, the flasks were placed in boiling water for 15 minutes for wax elimination, and then the heat cure Polymethyl methacrylate material was packed.

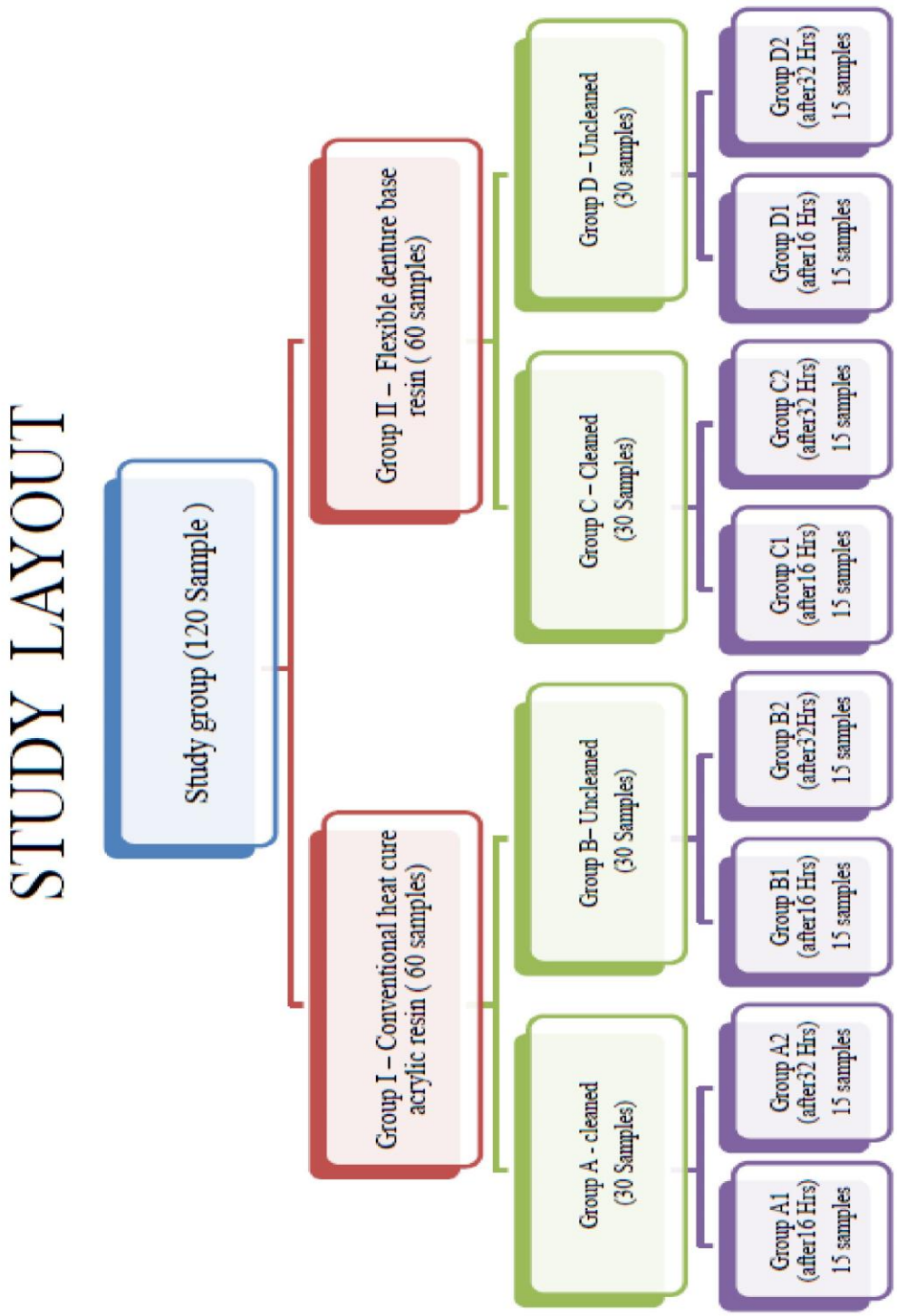
It was processed at 74^0C for approximately 2 hours and increasing the temperature of the water bath to 100^0C and processing for 1 hour and bench cooled for 30 minutes (rapid curing cycle)[1]. Deflasking was done and the acrylic samples were finished. Specimens were ground using progressively fine aluminium oxide papers (320, 400, and 600 grit). Final polishing was done using pumice slurry and felt wheel. All the above steps in specimen fabrication were done by the same operator. Finally they are stored in distilled water.

STERILIZATION

Before the procedure, all the specimens were subjected to Ethelene oxide sterilization and kept in a sterile disposable pouch.

PREPARATION OF CANDIDA ALBICANS CULTURE

Candida albicans MTCC strain was used for this study. Before each adhesion experiment 10 ml of Sabouraud's broth was incubated at 37^0C for 18 hours with Candida albicans. The broth was then plated onto Sabouraud's agar for purity checks. After purity checks, the candidal colonies were inoculated with 2 ml of sterile saline. A standardized cell suspension of candida was obtained by comparing the optical density with 0.5 Mcfarland solution, ensuring a concentration of 1 to 5 X 10^6cells/ml.


STUDY LAYOUT
Study group (120 Sample)
Group I – Conventional heat cure acrylic resin (60 samples)
Group II – Flexible denture base resin (60 samples)
Group A - cleaned (30 Samples)
Group B– Uncleaned (30 Samples)
Group C – Cleaned (30 Samples)
Group D – Uncleaned (30 samples)
Group A1 (after16 Hrs) 15 samples
Group A2 (after32 Hrs) 15 samples
Group B1 (after16 Hrs) 15 samples
Group B2 (after32Hrs) 15 samples
Group C1 (after16 Hrs) 15 samples
Group C2 (after32 Hrs) 15 samples
Group D1 (after16 Hrs) 15 samples
Group D2 (after32 Hrs) 15 samples

GROUP A1 - AFTER 16 HOURS (CLEANED): Conventional heat cure poly methyl methacrylate resin specimen (15 No.)-Subjected to mechanical brushing with denture cleansing paste before testing.

GROUP A2 – AFTER 32 HOURS (CLEANED): Conventional heat cure poly methyl methacrylate resin specimen (15 No.) - Subjected to mechanical brushing with denture cleansing paste before testing.

GROUP B1 - AFTER 16 HOURS (UNCLEANED): Conventional heat cure poly methyl methacrylate resin specimen (15 No.)-tested after 16 hours without cleaning.

GROUP B2 – AFTER 32 HOURS (UNCLEANED): Conventional heat cure poly methyl methacrylate resin specimen (15 No.)

GROUP C1 - AFTER 16 HOURS (CLEANED): Flexible denture base resin specimens (Valplast -15 No.) – Subjected to immersion in Valclean solution.

GROUP C2 - AFTER 32 HOURS (CLEANED): Flexible denture base resin specimens (Valplast -15 No.) – Subjected to immersion in Valclean solution before testing.

GROUP D1 – AFTER 16 HOURS (UNCLEANED): Flexible denture base resin specimens (Valplast- 15 No.)

GROUP D2 - AFTER 32 HOURS (UNCLEANED): Flexible denture base resin specimens (Valplast- 15 No.)

b. TESTING OF SAMPLES

PROCEDURE

2 ml of sterile artificial saliva (WET MOUTH -ICPA, INDIA) and 2 ml of the standardized cell suspension were added to each test tube. Each sample was placed in a sterile glass test tube in a vertical position and incubated statically (without agitation) at $37^{o}C$ for 16 hours. After 16 hours of incubation from the total 120 samples, 60 samples (30 PMMA, 30 Valplast) were removed. 15 samples of PMMA(Group A1) were subjected to mechanical brushing with denture cleansing paste (CLANDEN- DENT AIDS, INDIA), 15 samples of Valplast(Group c1) were immersed in chemical denture cleansing solution (VALCLEAN SOLUTION – VALPLAST CORPORATION, NEWYORK) and the remaining 15 samples from each group were stained without cleaning (Group B1 & Group D1).

The rest of the samples (60 samples) were left for another 16 hours of continuous incubation. The same procedure was repeated as described above to the remaining 30 samples of each

group (Group A2,C2 and Group B2,D2).After 32 hours the samples were subjected to staining.

CLEANSING PROCEDURE

VALPLAST SAMPLES

Valclean solution is prepared by dissolving 1 sachet of Valclean powder in 250 ml of warm water The samples were immersed in Valclean solution for 15 minutes. After 15 minutes the samples were removed from Valclean solution, washed, dried and stained. As per the manufacturer's instruction it can be stored for 7 days.

CONVENTIONAL POLYMETHYLMETHACRYLATE SAMPLES (DPI -INDIA)

The samples were cleaned with a tooth brush (STIM-Denture cleaning brush) & denture cleansing paste (CLANDEN – Dent Aids, INDIA)

STAINING PROCEDURE

Samples were dipped gently 3 times in 20 ml sterile PBS (Phosphate buffered saline) for 75 seconds to remove loosely attached cells, and left to dry. Next, the specimens were washed

with ethanol at 80% for 1 minute to fix the yeasts, and then stained for 1 minute with crystal violet. The adhered yeasts were counted using an optical microscope at 100x magnification under oil immersion. The sample is focused on one end and moved down in zigzag manner down, so that the entire length of the sample was examined. This procedure was repeated 3 times to avoid bias and repetition.

The results obtained were subjected to statistical analysis.

Fig 1: Valplast Cartridges

Fig 2: Conventional polymethyl methacrylate - DPI

Fig 3: Injection moulding machine

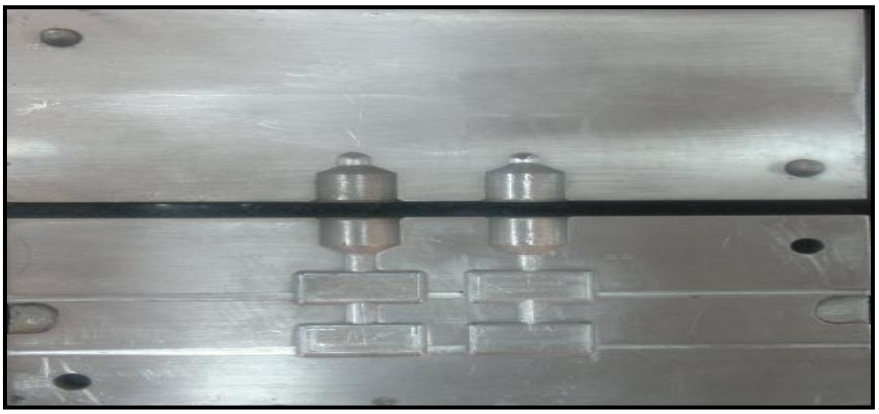


Fig 4: Metal mould

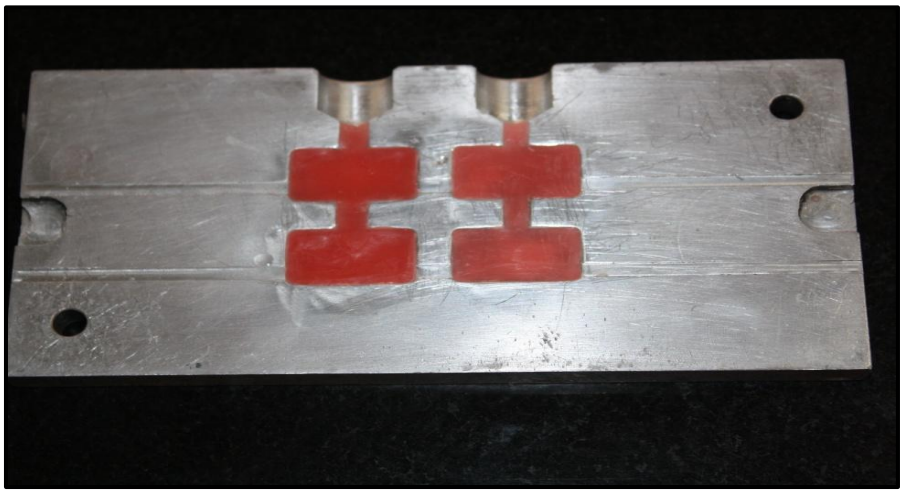


Fig 5: Preparation of wax pattern

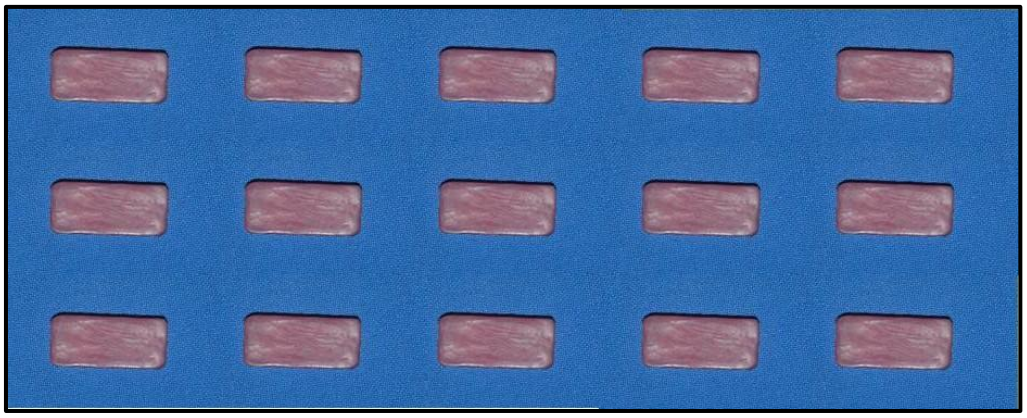


Fig 6: Valplast specimens

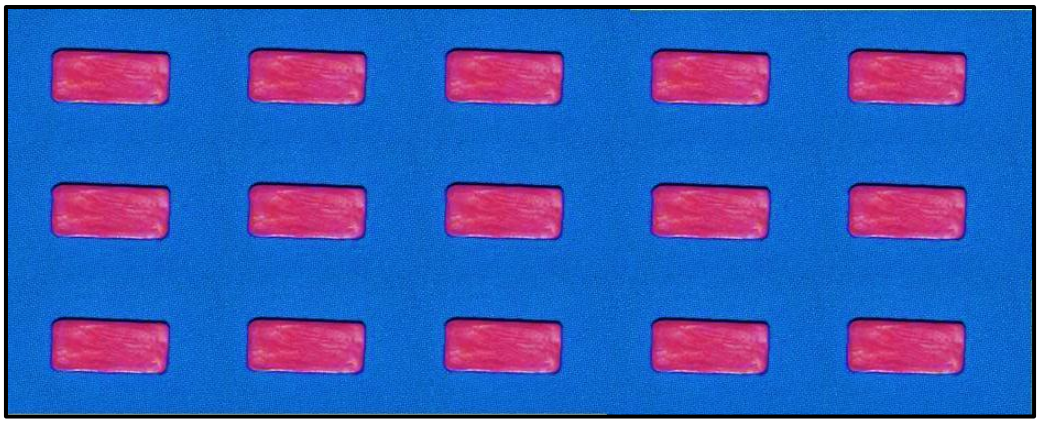


Fig 7: Conventional DPI specimens

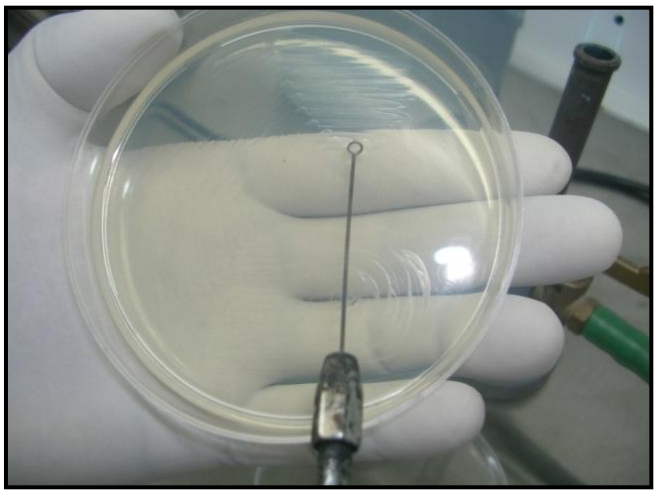


Fig 8: Preparation of candidal culture

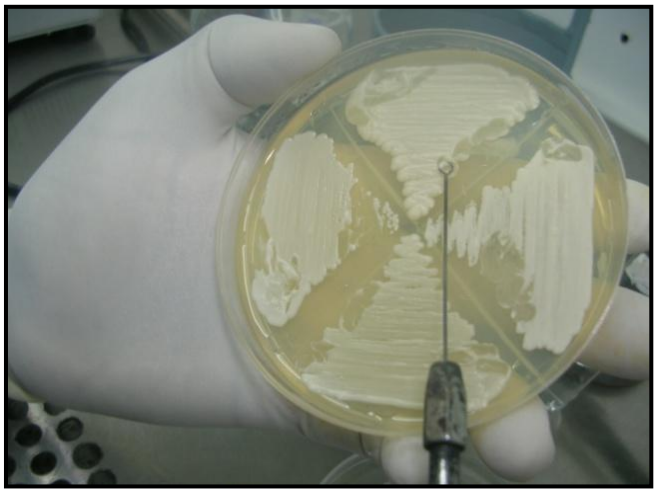


Fig 9: Candidal colonies

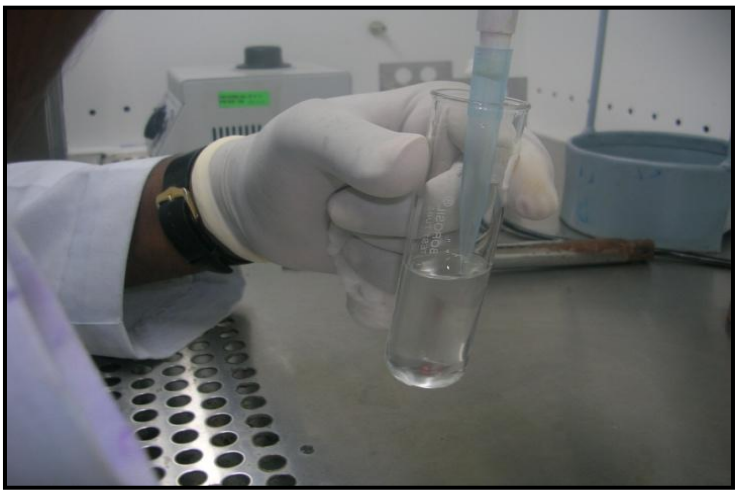


Fig 10: Preparation of standardized cell suspension

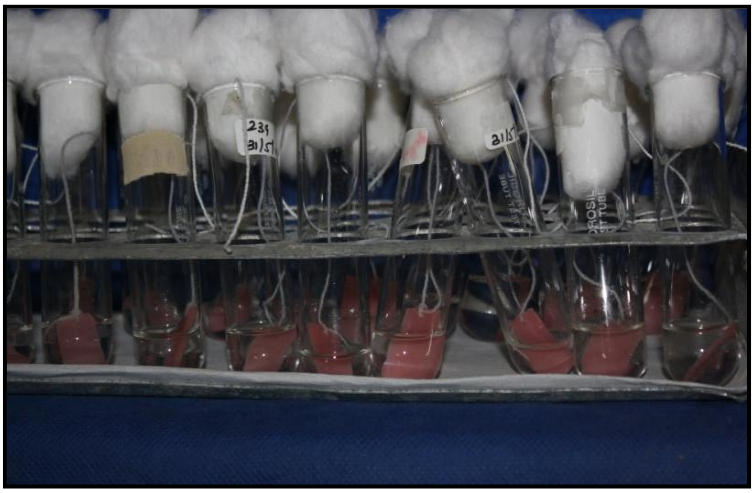


Fig 11: Specimens during incubation

Fig 12: Denture cleaning paste and brush

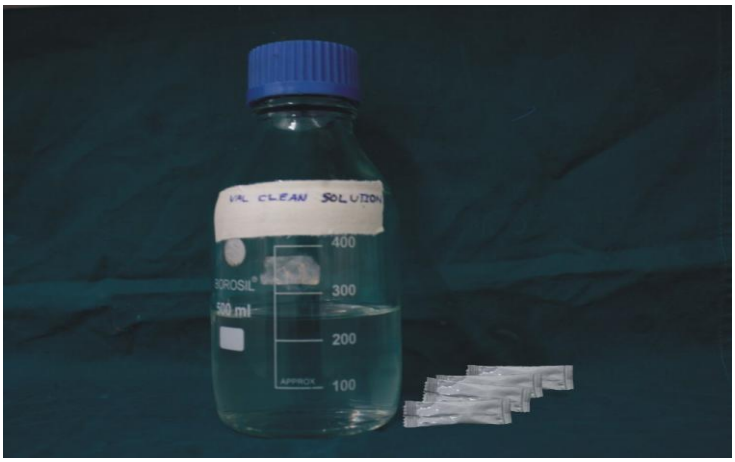


Fig 13: Valclean solution

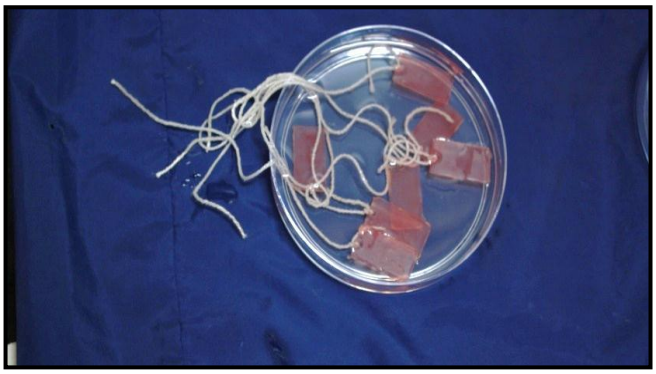


Fig 14: Staining procedure-step-1 wash in PBS

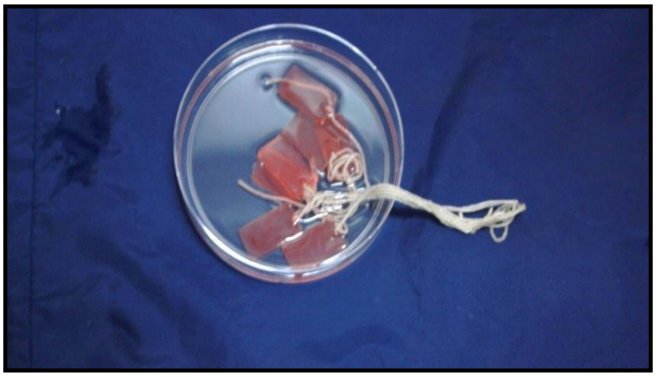


Fig 15: Step-2 wash in ethanol

Fig 16: Step-3 wash in crystal violet

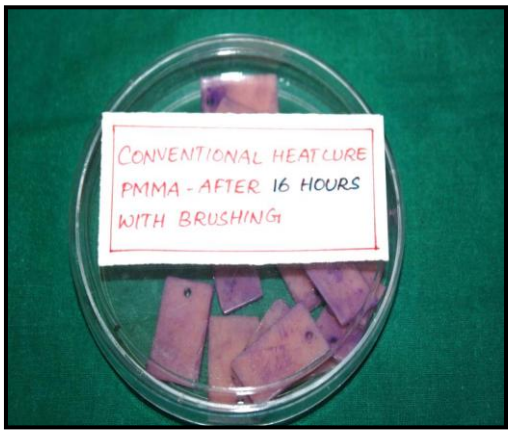


Fig : 17 - a

Fig: 17 – b

Fig :17 – c

Fig : 17 – d

Fig 17: Conventional PMMA specimens (DPI) after staining

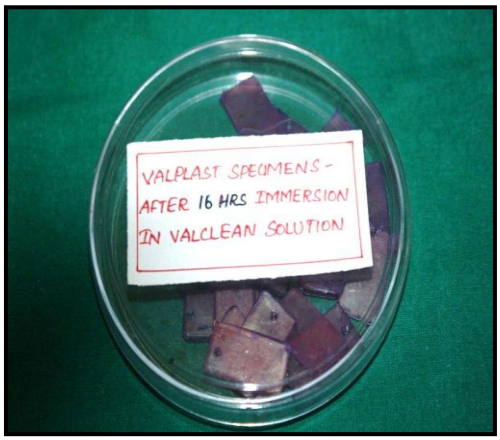


Fig : 18 - a

Fig: 18 – b

Fig : 18 - c

Fig : 18 - d

Fig 18: Valplast specimens after staining

Fig 19: Optical microscope

Candidal colonies in valplast under oil immersion

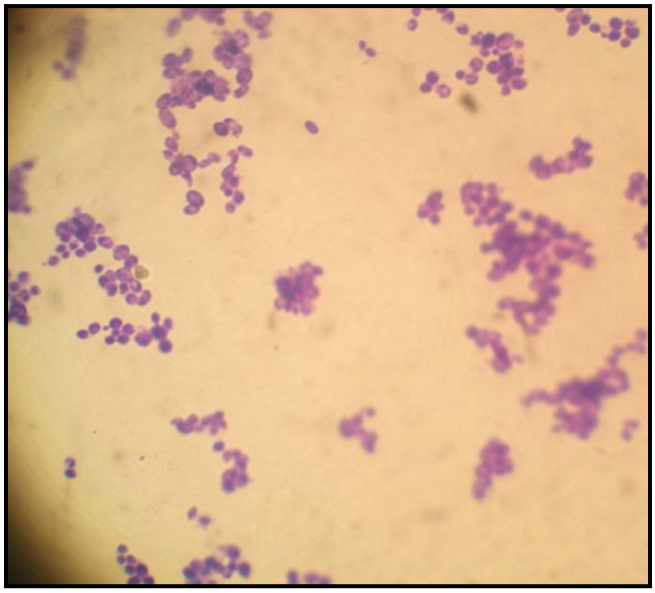


Fig 20: Before cleaning (with 100x magnification)

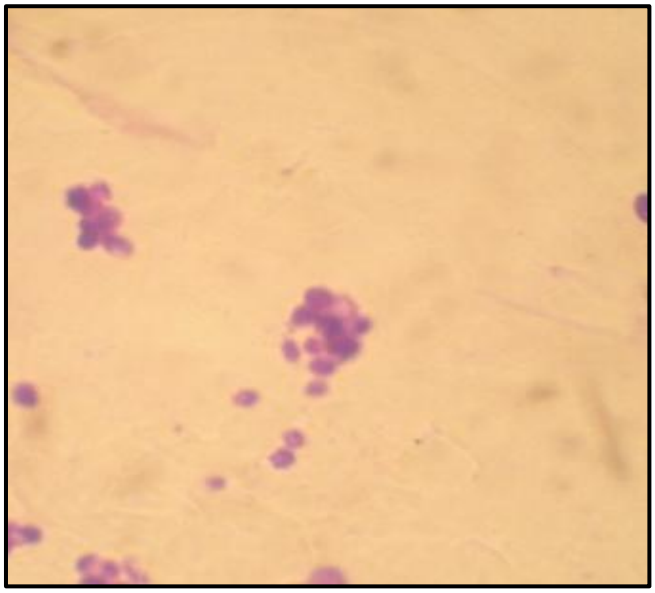


Fig 21: After cleaning (with 100x magnification)

Candidal Colonies in DPI Specimens Under Oil Immersion

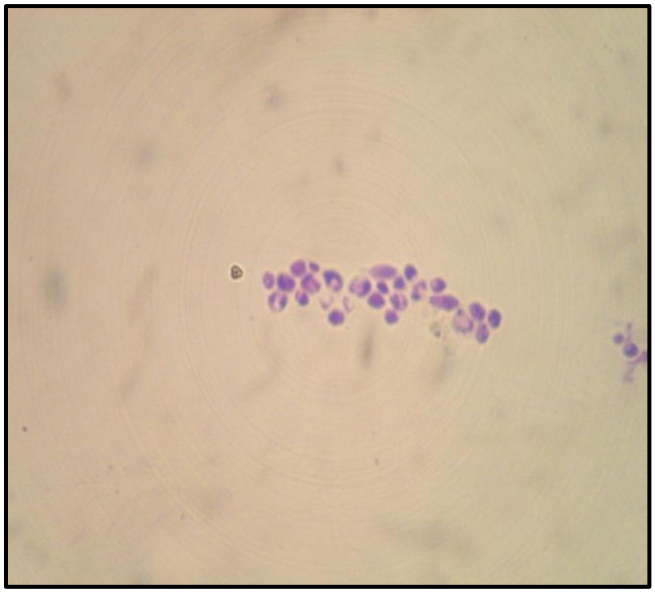


Fig 22: Before cleaning (with 100x magnification)

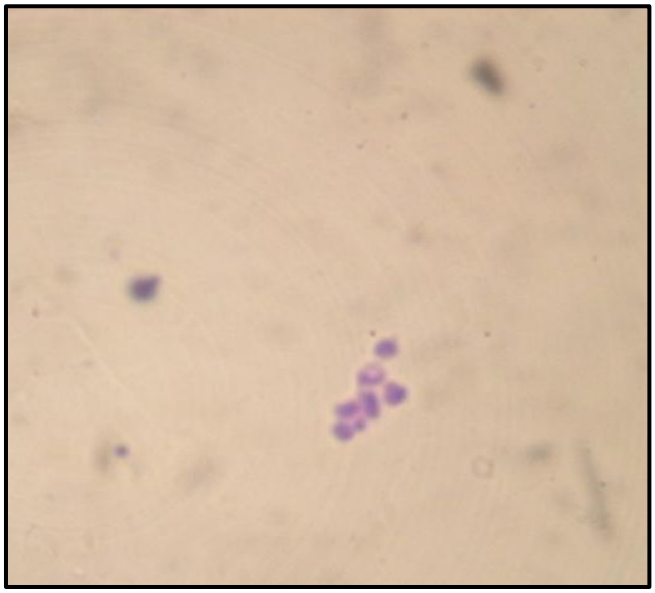


Fig 23: After cleaning (with 100x magnification)

5. Results

RESULTS

From the results obtained, the mean values were calculated. These results were subjected to statistical analysis. An independent sample t –test was done.

The t-test assesses whether the means of two groups are *statistically* different from each other. This analysis is appropriate to compare the means of two groups, especially for the two group randomized experimental design. It can be assumed that two distributions have the same variance.

The results were statistically analyzed to test the following hypothesis.

HYPOTHESIS: "*There is no difference in candidal adherence between flexible denture base resin and conventional Polymethyl methacrylate acrylic resin*".

Table -1 Statistical analysis to compare the No. of organism adhered on uncleaned Denture base resin samples of Valplast and Conventional PMMA after 16 Hours

Group Statistics

Denture base material (16Hrs uncleaned)	N	Mean	Std. Deviation	Std. Error Mean
Valplast	15	20.4667	6.25490	1.61501
PMMA	15	2.4667	.83381	.21529

Independent Samples test

16 Hours (uncleaned)	t-test for Equality of Means				
	t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference
Equal variances assumed	11.048	28	.000	18.0000	1.62930
Equal variances not assumed	11.048	14.497	.000	18.0000	1.62930

This table shows that the mean No. of organism in Valplast and conventional PMMA after 16 hours (uncleaned samples) were 20.4667 and 2.4667 and the standard deviation were 6.254 and 0.83381 respectively. The P value is significant (P<.001). It can be inferred that in Valplast material the No. of adhered organism is more compared to Conventional PMMA.

Table - 2. Statistical analysis to compare the No. of organism adhered on cleaned Denture base resin samples of Valplast and Conventional PMMA after 16 Hour

Group Statistics

Denture base material 16Hrs(cleaned)	N	Mean	Std. Deviation	Std. Error Mean
Valplast	15	9.333	1.16292	.30026
PMMA	15	.2000	1.41404	.10690

Independent Samples test

16 Hours (Cleaned)	t-test for Equality of Means				
	T	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference
Equal variances assumed	30.538	28	.000	9.73333	.31873
Equal variances not assumed	30.538	17.493	.000	9.73333	.31873

This table shows an increase in no of organism adhered to valplast denture base with the mean and standard deviation of 9.9333and 1.16292 respectively, when compared to PMMA with the mean and standard deviation of 0.200 and 1.41404 . There was a significant increase(P<.001) in adherence of Candida colonies on cleaned valplast denture base after 16 hours.

Table – 3. Statistical analysis to compare the No. of organism adhered on uncleaned Denture base material made of Valplast and Conventional PMMA after 32 Hours

Group Statistics

Denture base material (32 Hrs uncleaned)	N	Mean	Std. Deviation	Std. Error Mean
Valplast	15	117.1333	10.31550	2.66345
PMMA	15	6.7333	2.28244	.58932

Independent Samples Test

32 Hours (Uncleaned)	t-test for Equality of Means				
	t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference
Equal variances assumed	40.471	28	.000	110.40000	2.72787
Equal variances not assumed	40.471	15.368	.000	110.40000	2.72787

In the table 3 the mean No. of organism in Valplast and conventional PMMA after 32 hours (uncleaned samples) were 117.133 and 6.7333respectively. It shows the P value ($P< 0.001$) is significant. It can be inferred that the number of organism in Valplast is more when compared to Conventional denture base material after 32 hours of uncleaned samples.

Table – 4. Statistical analysis to compare the No. of organism in cleaned Denture base material samples of Valplast and Conventional PMMA after 32 Hours

Group statistics

Denture base material (32 Hrs cleaned)	N	Mean	Std. Deviation	Std. Error Mean
Valplast	15	6.4667	1.50555	.38873
PMMA	15	.1333	.35187	.09085

Independent Samples Test

32 Hours (cleaned)	t-test for Equality of Means				
	t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference
Equal variances assumed	15.865	28	.000	6.33333	.39921
Equal variances not assumed	15.865	15.525	.000	6.3333	.39921

This table shows an increase in the mean No. of organism in valplast (6.4667) than the conventional PMMA (1.333) after 32 hours (cleaned samples) with the standard deviation of 1.50555 and .351 respectively. P value is significant (P<0.001). It can be inferred that in Valplast the No. of organism is more when compared to conventional denture base material. From the results obtained from table 1 to 4 the proposed hypothesis is rejected.

Table - 5. T-Test for the Mean difference in the No. of organism in cleaned and uncleaned Denture base material samples of Valplast after 16 Hours & 32 Hours

Group Statistics

Denture base material		N	Mean	Std. Deviation	Std. Error Mean
Valplast 16Hrs	Cleaned	15	9.333	1.16292	.30026
	Uncleaned	15	20.4667	6.25490	1.61501
Valplast 32 Hrs	Cleaned	15	6.4667	1.50555	.38873
	Uncleaned	15	117.1333	10.31550	2.66345

Independent Samples Test

Denture base material		t-test for Equality of Means				
		t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference
Valplast 16 hours	Equal variances assumed	-6.412	28	.000	-10.53333	1.64268
	Equal variances not assumed	-6.412	14.967	.000	-10.53333	1.64268
Valplast 32hours	Equal variances assumed	-41.115	28	.000	110.66667	2.69167
	Equal variances not assumed	-41.115	14.596	.000	110.66667	2.69167

In the table 5 the mean value of Valplast materials after 16 hours both before cleaning and after cleaning were 20.667 and 9.333 respectively. And after 32 hours of incubation the uncleaned and cleaned Valplast material showed the mean adherence of 117.1333 and 6.4667 respectively. The P value is significant P (<.001). It can be inferred that the number of organism is significantly reduced on Valplast samples after cleaning.

Table - 6. T-Test for the Mean difference in the No. of organism in cleaned and uncleaned Denture base material samples of conventional PMMA after 16 Hours & 32 Hours

Group Statistics

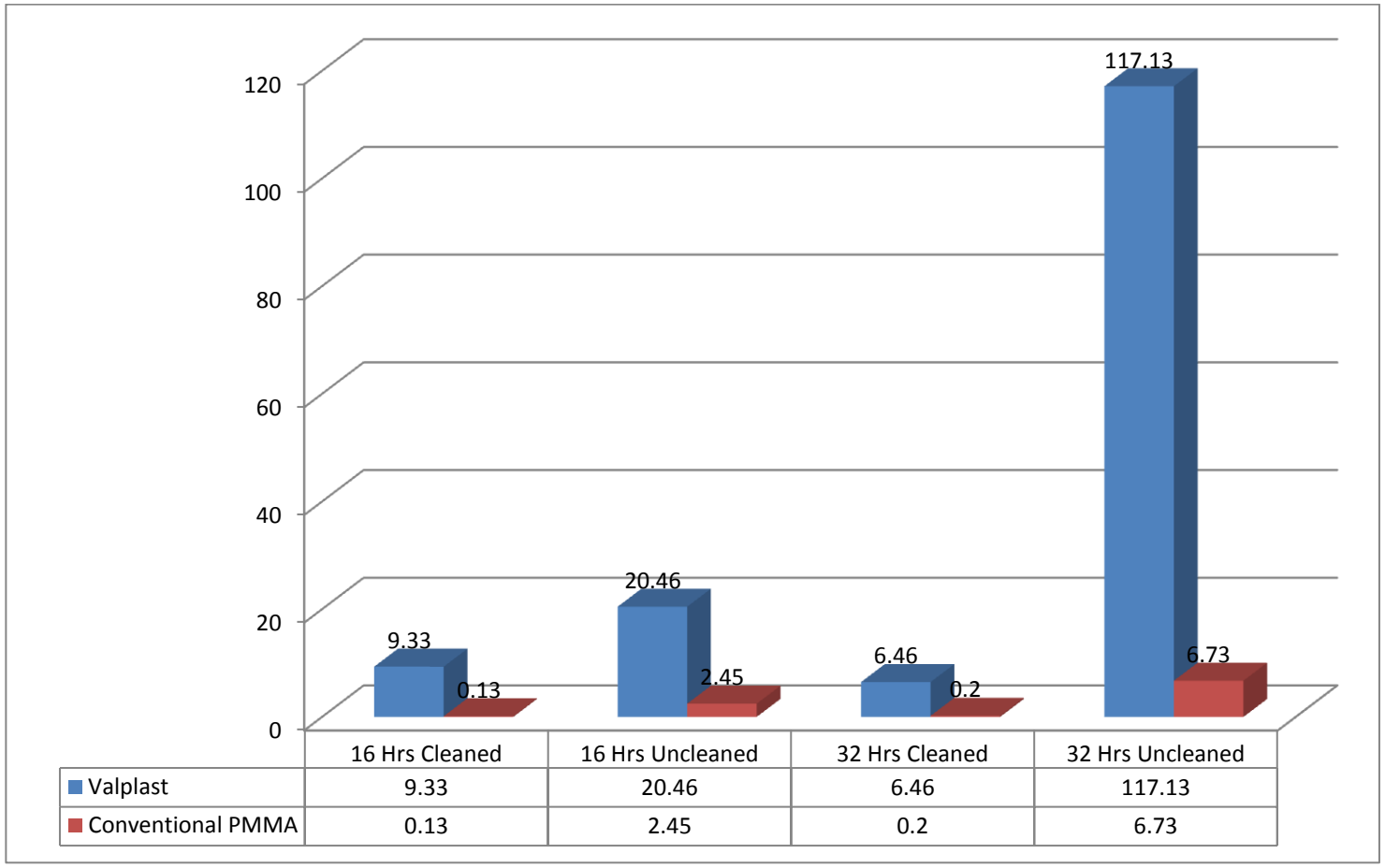
Denture base material		N	Mean	Std. Deviation	Std. Error Mean
Conventional PMMA16Hrs	Cleaned	15	.2000	.41404	.10690
	Uncleaned	15	2.4667	.83381	.21529
Conventional PMMA 32 Hrs	Cleaned	15	.1333	.35187	.09085
	Uncleaned	15	6.7333	2.28244	.58932

Independent Samples Test

Denture base material		t-test for Equality of Means				
		t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference
Conventional PMMA 16Hrs	Equal variances assumed	-9.430	28	.000	-2.26667	.24037
	Equal variances not assumed	-9.430	20.508	.000	-2.2667	.24037
Conventional PMMA 32 hrs	Equal variances assumed	-11.069	28	.000	-6.60000	.59628
	Equal variances not assumed	-11.069	14.665	.000	-6.60000	.59628

In the table 6 the mean value of conventional PMMA samples after 16 hours both before cleaning and after cleaning were 2.4667 and 0.2000. And after 32 hours of incubation the uncleaned and cleaned conventional PMMA samples showed the mean adherence of 6.733 and 0.1333. The P value is significant (<.001).It can be inferred that the number of adhered organism is significantly reduced on conventional PMMA after cleaning.

CHART NO. 1. THE COMPARISON OF CANDIDAL ADHERENCE BETWEEN CLEANED AND UNCLEANED SAMPLES OF FLEXIBLE AND CONVENTIONAL PMMA RESINS



	16 Hrs Cleaned	16 Hrs Uncleaned	32 Hrs Cleaned	32 Hrs Uncleaned
Valplast	9.33	20.46	6.46	117.13
Conventional PMMA	0.13	2.45	0.2	6.73

6. Discussion

DISCUSSION

Candidal species like Candida albicans, C. dubliniensis[11], C.glabrata[23] and C.tropicalis[30] are present in normal oral flora. These organisms become pathogenic in patients, who are immuno compromised, with poor oral hygiene and with xerostomia[1]. Denture stomatitis is an inflammatory condition of the palatal mucosa commonly seen in patients wearing the prosthesis day and night continuously without removing and cleaning. Microbial adhesion on the denture as a result of continuous wear causes irritation to the palatal mucosa. The prevalence of myceliated colonies of C albicans isolates in denture wearer with denture stomatitis is 77.5%[7]. The functional deficiency of (phagocytosis) neutrophils and aging alters the characters of neutrophil cells which facilitate the establishment of Candida related-denture stomatitis[12]. Candidal colonization in denture is more frequently associated with diabetic patients. Elimination of local and systemic factors and improving oral hygiene with effective post denture maintenance decreases the incidence of candidal colonization[20].

An essential prerequisite for Candidal colonization and infection is the ability of yeast to adhere on superficial epithelial cells as well as to the fitting surface of the denture, which acts as a reservoir of infection[35]. Multiplication of fungi is limited to the

mucosal surfaces, and any weakening of surface host defence, will allow the fungal elements[19] to penetrate into the tissues, blood and can spread to adjacent organs. Translocation of C. albicans occurs through direct penetration of enterocytes, different from classical phagocytosis. Internalization of the Candida occurs due to the disturbance of the plasma membrane and brush border[18]. So it becomes important to find the adhesion of candida in the denture base which is constantly in contact with the mucosa.

The factors that could influence the adhesion of candida in the denture base are the surface roughness, material composition and cleaning methods used.

Earlier numerous studies have been conducted to evaluate the adherence of candida in different acrylic resin base. Heat cure polymethylmethacrylate denture base resins showed less adherence of Candidal cells compared to self cure and light cure resins[33]. Based on the above findings, this study was carried out to evaluate the adherence potential of Candida albicans on flexible denture base resin (Valplast). Although valplast material is used from 1950's, there are no studies present on the adherence potential of candida to the material. This study was conducted to assess the above mentioned characteristic.

A total of 120 samples, 60 flexible denture base (Valplast) and 60 samples of conventional heat cure Polymethyl methacrylate resin material (DPI) were prepared as per manufacturer's instructions. All the samples were stored in distilled water.

All the samples were immersed in standardized cell suspension of candida albicans and incubated for 16 hours and 32 hours. The 60 samples (30 flexible and 30 PMMA samples) were removed and subjected to mechanical and chemical cleaning, and the other half was used for the study without cleaning at 16 hours and 32 hours respectively. The samples were stained and observed under optical microscope in oil immersion with 100x magnification. The results obtained were subjected to statistical analysis using independent sample T test.

It was observed that after 16 hours of incubation, uncleaned samples of valplast showed more adherences with the mean of 20.4667 than the uncleaned samples of conventional Polymethyl methacrylate with the mean of 2.4667(Table-1). After cleaning, the mean adherences were reduced on valplast with the mean of 9.333 and conventional Polymethyl methacrylate resin (DPI) samples with the mean of .2000(Table-2). According to Table-3, after 32 hours of incubation, uncleaned samples of valplast showed more adherences with the mean of 117.1333 than the conventional Polymethyl

methacrylate resin mean of 6.733. Table-4 showed cleaning after 32 hours, reduced the number of colonies on both valplast samples and conventional Polymethyl methacrylate samples with the Mean of 6.4667 and .1333 respectively. Considering all the above tables it can be inferred that there is a significant increase in candidal adherence on valplast when compared to conventional PMMA.

The reason for fewer adherence of candida on conventional PMMA may be probably due to its residual monomer[9] content, the attractive Vander Waals forces or hydrophobic forces and electrostatic forces[21].

Waltimo et al[43] showed fewer adherences on newly polymerized resin than the water stored denture base polymer and said that the salivary pellicle reduce the hydrophobicity. The sustained release of residual monomer and their oxidation may inhibit the initial adherence of yeast on the surface of the acrylic resins. **Park et al [31]**showed that increase in monomer content from 5% -10% resulted in fewer adherences of candida, but this lead to reduction in transverse and flexural strength[31].

Table-5 shows that uncleaned Valplast and conventional samples have more adherences in 32 hours with the mean of 117.133 and 6.7333 respectively than in 16 hours with the mean of 20.466

and 2.2667 respectively. Within the cleaned Valplast samples studied, Valplast samples after 32 hours showed fewer adherences in 32 hours (mean of 6.4667) than the 16 hours (mean of 9.333) samples. Table 6 shows uncleaned conventional samples showed more adherences after 32 hours (mean-6.7333) than in 16 hours (mean-2.2667). The adherence of candida colonies in 16 hour and 32 hour cleaned samples were not statistically significant.

Different methods are used for cleaning dentures like brushing, use of paste and powders, ultrasonic agitation, chemical cleansing using peroxides, hypochlorite, disinfecting agents and enzymes.The most common method of denture cleansing is brushing with toothpaste and it is very effective for removing artificial discolorations and plaque from acrylic resin dentures[8]. The powder and paste products containing insoluble calcium carbonate are highly abrasive than the dentifrices containing soluble sodium bicarbonate. Ultrasonic agitation is an efficient method for the removal of denture plaque when disinfectant solutions are used[8].

Chemical cleaning methods are based on alkaline peroxide and alkaline hypochlorite solutions. Peroxide cleansers seem to be most effective on new plaque and stains when the denture is soaked in the chemical solution for several hours or overnight. These products are not effective than brushing with soap[6]. Alkaline

hypochlorites are useful as denture cleansers, because they remove stains, dissolve mucin and other organic substances and are bactericidal and fungicidal. It is believed that hypochlorite acts directly on the organic matrix of plaque, causing dissolution of the polymer structure but does not dissolve calculus. It may inhibit calculus formation on dentures by dissolving the plaque organic matrix[6]. Dentures immersed in 0.5% Sodium hypochlorite for 10 minutes removed Candida spp. biofilm efficiently than the alkaline peroxide denture cleansers in from denture liner surfaces and prevented biofilm recolonization[41]. The roughness of the resins increased after the cleaning, but the difference among the cleansers was not statistically significant. The lowest amount of biofilm formed on acrylic resin specimens was with NaOCl [24].

Denture cleansers contain chelating agent ethylene diamine tetra acetic acid (EDTA)) and mixture of enzymes (papain, lipase, amylase, trypsin), enzymes like proteinase and mutanase and the yeast lytic enzymes were found to be effective in removing mucin, heavy deposits of calculus and reduction and formation of new plaque on dentures. The cleansers were also bactericidal and fungicidal. This emphasis that the c.albicans is adhered to the acrylic resin surfaces by protein[38].

2% chlorhexidine gluconate[25], 0.12% digluconate chlorhexidine[3], and self-cured poly methyl methacrylate (PMMA) acrylic resin mixed with 10% w/w chlorhexidine powder reduced the adherence of candida species to acrylic resins. The inhibition of yeast adherence by chlorhexidine persisted for up to 19 days after the exposure of the acrylic strips to the disinfectant[27]. The drug released also demonstrated antifungal activity against the resistant strain of C. Albicans[34].

The chemical composition of the Valplast cleaner is undisclosed and the samples were immersed only for 15 minutes as per the manufacturer instructions in this study. The Valplast cleaned samples after 32 hours and 16 hours showed reduced number of colonies with the mean of 6.4667 and mean of 9.333 respectively.

The conventional Polymethyl methacrylate cleaned samples after 32 hours and 16 hours showed mean of .1333 and mean of .2000 respectively.The cleaning methods reduced the colonies but did not eliminate the organisms on both the materials. The Valplast and PMMA samples showed significant reduction in organisms between cleaned and uncleaned samples.

Studies show that mechanical cleansing with the tooth brush and paste is effective than the chemical cleaning[30]. The time of

immersion in denture cleanser solution have to be evaluated to eradicate the microbial colonies from the denture base.

The surface free energy [28] as indicated, by which saliva spreads over a surface, was one of the factors for adherence of candida. The variations in surface energy that resulted from differences in the composition of the different PMMA resins appear to have no influence on the adhesion of C. albicans, and denture stomatitis[14]. Another study by **Hashiguchi M et al**[16] resulted in overall reduction of the microorganism in 1.00–4.50% glycine-type amphoteric surfactant solutions than the commercial enzymatic denture cleaner (Polident). The results suggested that glycine-type amphoteric surfactant solution may be effective as a denture cleaner in conjunction with an ultrasonic cleaning device[16].

In addition to the above factors, surface characteristics such as micro porosities and roughness may cause the surface to harbor microorganisms that are difficult to remove by mechanical or chemical cleansing, thus increasing microorganism adherence in vitro[29]. An increase in surface roughness facilitated yeast retention on silicone, acrylic resin surfaces[40], cold and heat polymerized soft liner[42].

The growth on surfaces is a natural part of the Candida lifestyle and its colonization in denture users are expected[29]. In contrast, when the influence of polymerization methods of acrylic resins and human whole saliva was studied on the adherence to acrylic resin surfaces, saliva was capable of reducing the adherence of Candida species and the surface roughness and free energy did not influence the adherence rates. Presence of an immobilised salivary coating had little effect on either the candidal adhesion or biofilm formation. The addition of saliva to the incubation medium quantitatively affected biofilm formation especially on day 3 and day 4, without any significant effect on yeast adhesion[44].

Studies have proved that the human saliva is capable of reducing fungal colonies[26, 29] and the salivary pellicle formation precipitates candidal colonization[29]. In this study the salivary substitute, the artificial saliva (WET MOUTH-ICPA) contained Glycerine and sodium carboxy methyl cellulose. This solution did not contain any antifungal agents.

In this study the surface property of the flexible material was not accounted, as our primary aim was to check the adherence of micro organisms to the material. A detailed study has to be established on the surface smoothness of the flexible material.

In our study, the conventional PMMA showed fewer adherences than the Valplast both in uncleaned and cleaned conditions. The new polyamides are easily colonized by Candida species and may present a convenient substratum for microbial colonization[9] like the conventional PMMA.

In this study, time intervals of 16 hours and 32 hours were taken into consideration, simulating the patients cleaning the denture every night, or wearing the dentures continuously. The result of this study shows that when the patient wears the prosthesis overnight, the candidal adherence increases. The chemical cleanser reduced the numbers on Valplast samples but did not completely eliminate the colonies. **Dills et al**[5] recommended denture cleansers along with brushing for proper denture hygiene.

Fernandes et al[8] suggested to use denture brushes for effective removal of biofilm to reduce candidal adherence. The Valplast manufacturer recommends to avoid brushing and use a denture cleanser (Valclean solution) provided by them. Continuous brushing can produce abrasion and exposure of the fibers; weakening the base and increasing the surface roughness[36]. The denture cleansers can produce color changes, increased surface roughness and diminish the flexural strength of the acrylic resin[24].

Li et al[23] concluded that the daily use of cleanser solutions in clinical practice may promote a population shift towards non-albicans species, such as C. glabrata which is associated with systemic infections and having a high mortality rate.

The microscopic evaluation in this study was conducted by conventional optical microscope (100 x magnifications) under oil immersion which allows only manual count. There are chances for human error in accounting the number of organisms. A study by scanning electron microscope would be preferable to find out the adherence and surface roughness.

In this study two commonly used materials were tested. However, the reason for more adherences of candida on flexible denture base resin has to be studied extensively including the surface properties like roughness and porosities, material composition, effectiveness of the cleanser solution, devices and simulation of oral conditions. Various flexible materials can be included in the studies in future and research should be necessary based on above factors.

7. Summary and Conclusion

SUMMARY AND CONCLUSION

This study was carried out to evaluate and compare the adherence potential of Candida albicans on flexible denture base material (Valplast) and conventional heat cure Polymethyl methacrylate (DPI).

A total number of 120 samples, 60 in flexible denture base material and 60 samples in conventional heat cure polymethyl methacrylate material were prepared according to manufacturer's instructions. Each sample was immersed in standardized cell suspension of candida albicans, with artificial saliva. After 16 hours and 32 hours of incubation, samples were stained and observed under optical microscope before and after cleaning. Cleaning was done by mechanical brushing and cleanser solution as per the manufacturer's instructions.

Within the limitations of this study the following conclusions can be drawn,

1. Flexible denture base resin and conventional PMMA resin sample showed candidal adherence after 16 and 32 hours, but the valplast showed more adherence than the conventional PMMA resin.

2. Mechanical cleaning on Conventional sample showed fewer adherences when compared to flexible denture base resin cleaned in valclean solution.
3. Candidal colonies after cleaning showed significant reduction, compared to the uncleaned samples of both conventional PMMA and flexible denture base resin materials.

The residual monomer release, surface properties like free surface energy, polishing, surface irregularities like roughness, porosities and growth on human saliva were not evaluated in this study. The results were evaluated with optical microscopy only, an additional electron microscopy and profilometer studies would have given enhanced results. Although several studies have been attempted on different properties, an extended study has to be made to evaluate all the properties in one study, to understand the increased level of adherences to polyamide resins.

8. Bibliography

BIBLIOGRAPHY

1. **Anusavice K J,** Philips science of dental materials, Eleventh edition, 143-166, Elsevier Inc 2008.
2. **Barry Shipman**. Clinical evaluation of oral Candida in cancer chemotherapy patients. J Prosthet dent 1979; 49: 63-67.
3. **Cervantes F A, Garcia de Sousa, Paradella T C, Koga-Ito CY, Jorge A O C**. Effect of sodium bicarbonate on Candida albicans adherence to thermally activated acrylic resin. Braz Oral Res 2009; 23: 381-5.
4. **Chladek G, Mertas A, Barszczewska-Rybarek I, Nalewajek T, Żmudzki J, Król W, Łukaszczyk J**. Antifungal Activity of Denture Soft Lining Material Modified by Silver Nanoparticles - A Pilot Study. Int J Mol Sci 2011; 12: 4735-4744.
5. **Dills S S, Olshan A M, Goldner S, Brogdon C**. Comparison of the antimicrobial capability of an abrasive paste and chemical-soak denture cleaners. J Prosthet Dent 1988; 60: 467-470.
6. **Ejvind Bud and J Brgensen.** Materials and methods for cleaning dentures. J Prosthet Dent 1979; 42: 619-623.
7. **Emami E, Seguin J, Rompre P H, Kodninck L, Grandmont P, Jean Barbeau**. The relationship of myceiliated colonies of candida albicans with denture stomatitis: An in vivo/In vitro study. Int J Prosthodont 2007; 20: 514-520.

8. **Fernandes R A,Lovato-silva C H, Paranhos H F, Izabel Yoko.** Efficacy of three denture brushes on biofilm removal from complete denture. J Appl Oral Sci 2007; 15: 39-43.
9. **Fernandes FS, Tatiana Pereira- Cenci, da Silva WJ, Ricomini Filho AP, Straioto FG, and Del Bel Cury A A,** Efficacy of denture cleansers on Candida spp. biofilm formed on polyamide and polymethyl methacrylate resins. J Prosthet Dent 2010; 105: 51-58.
10. **Figueiral M H, Azul A, Pinto E, Fonseca P A, Branco F M and Scully C.** Denture-Related Stomatitis: Identification Of etiological and predisposing Factors – A Large Cohort. J Oral Rehab 2007; 34: 448–455.
11. **Gasparaoto, Dionı´sio T J, Oliveira C E, porto V C, Gelani V,Santos C F, campaneli A P, Lzlara V S**. Isolation of candida dubliniensis from denture wearers. J med micro 2009; 58: 959–962.
12. **Gasparoto T H, Vieira, Campanelli A P, Carvalho Porto, Lara V S**. Ageing exacerbates damage of systemic and salivary neutrophils from patients presenting Candida-related denture stomatitis. Immunity & Ageing 2009; 6: 3.
13. **Goiato MC, Santos DM, Haddad MF, Pesqueira AA.** Effect of accelerated aging on the microhardness and color stability of flexible resins for dentures, Braz Oral Res 2010; 24: 114-9.

14. **Granger C S, Lapiedra R C, Trapero J C, Highsmith J D**. In vitro study of the Adherence of candida Albicans to Acrylic resins: Relationship to surface energy. Int J Prosthodont 2005; 18: 392-398.

15. **Gunjan Dhir, David W. Berzins, Dhuru V B, A. Raj Periathamby and Andrew Dentino.** Physical Properties of Denture Base Resins Potentially Resistant to Candida Adhesion. J Prosthodont 2007; 16: 465–472.

16. **Hashiguchi M, Yasuhiro nishi, Kanie T, Seiji Ban, Nagaoka E**. Bactericidal efficacy of glycine-type amphoteric surfactant as a denture cleaner and its influence on properties of denture base resins. Dent Mater J 2009; 28: 307-314.

17. **Jacob John, Shivaputrappa A. Gangadhar, and Ila Shah,** Flexural strength of heat-polymerized polymethyl methacrylate denture resin reinforced with glass, aramid, or nylon fibers. J Prosthet Dent 2001; 86: 424-7.

18. **Jean-Marcel Senet,** Candida adherence phenomena, from commensalism to pathogenicity. Int microbiol 1998; 1: 117–122.

19. **Jeniel E Nett, Marchillo K, Spiegel C A, Andes D R**. Development and Validation of an In Vivo Candida albicans Biofilm Denture Model. Infect Immun 2010; 78: 3650-3659.

20. **Kamran M H L, Jafari AA, Abbas Falah-Taft, Tavakoli E, Falahzadeh M H**. Introduction Candida Colonization on the

Denture of Diabetic and Non-diabetic Patients. Dent Res J 2009; 6: 23-27

21. **Klotz S A, David J D, James E. Zajic**. Factors governing adherence of Candida species to plastic surfaces. Infect immun. 1985; 50: 97-101.
22. **Leonard Garth Lowe**. Flexible denture flanges for patients exhibiting undercut tuberosities and reduced width of the buccal vestibule: A clinical report. J Prosthet dent 2004; 92: 128-31.
23. **Li L, Reading S, Dongari Bagtzoglou A.** candida glabrata; an emerging oral opportunistic pathogen. J Dent Res 2007; 86: 204-15.
24. **Lima J S, Moura F M C X, Del bel curry A A, Garcia R C M R, Cury J A.** Effect of enzymatic and NaOCl treatments on acrylic roughness and on biofilm accumulation. J oral rehabil 2006; 33: 356-362**.**
25. **Mac courtie J, W Macfarlane, Samaranayake.** Effect of chlorhexidine gluconate on the adherence of candida species to denture acrylic. J Med Microbiol 1985; 20: 97-104.
26. **Mc courtie J, Julia Douglas**. Relationship between cell surface composition of candida albicans and adherence to acrylic after growth on different carbon sources. Infect Immun 1981; 32: 1234-1241.

27. **Mc courtie J, Macfarlane TW, Samaranayake LP**. Effect of saliva and serum on the adherence of candida species to chlorhexidine-treated denture acrylic. J Med Microbiol 1986; 2: 209-2I3.

28. **Minagi S, IMiyakey, Iinagaki K, Ttsuru H, Suginaka H**. Hydrophobic interaction in candida albicans and candida tropicalis adherence to various denture base resin materials. Infect Immun 1985; 47: 11-14.

29. **Moura J S, Silva WJ, Tatiana Pereira, Del Bel Cury A A, and Rodrigues Garcia R C M,** Influence of acrylic resin polymerization methods and saliva on the adherence of four Candida species, J Prosthet Dent 2006; 96: 205-11.

30. **Paranhos HO, Silva-lovato C H, De Souza R F, Patricia C, Freita-Pontes, Watanabe E, Izabel Y.** Effect of Three Methods for cleaning denture on biofilms formed in vitro on Acrylic resin. J Prosthodont 2009; 18: 427-431.

31. **Park S, Maggie Chao, P. A. Raj**. Mechanical Properties of Surface-Charged Poly (Methyl Methacrylate) as Denture Resins. Int J dent 2009; 1- 6.

32. **Peracini A,Resende Davi L, Ribeiro N, Desouza R F, Lovato da silva, Paranhos**. Effect of denture cleansers on physical properties of heat-Polymerized acrylic resin. Journal of Prosthodontic Research 2010; 54: 78-93.

33. **Rajani Kalla, Harikesh Rao, Sunil Kumar MV**. Surface adherence of Candida Albicans to different Polymethyl methacrylate Denture Base Resins: An in-vitro-study. Int J Prosthetic Dentistry 2011; 2: 1: 2-7.

34. **Ryalat S, Darwish R,Wala Amin**. New form of administering chlorhexidine for treatment of denture-induced stomatitis. Therapeutics and Clinical Risk Management 2011; 7: 219–225.

35. **Samaranayake LP.** Chronic hyperplastic candidiasis/candidiosis (Candidal leukoplakia)**,** Crit Rev Oral Biol Oral Med 2003; 14: 253-267.

36. **Shamnur SN, Jagadeesh KN, Kalavathi, Kashinath**. Flexible dentures – an alternate for rigid dentures?. J Dent Sci & Res 2011; 1: 74 – 79.

37. **Stern M N**. Esthetic retention for modern dental prosthesis. N.Y.state D J 1964; 30.

38. **Tamamoto M, Hamada T, Miyake Y, and Suginaka H**, Ability of enzymes to remove Candida. J Prosthet dent 1985; 53: 214-216.

39. **Thirumurthy V R, Bindhoo Y A, Sunil Joseph Jacob, Anjana Kurien, Limson K S.** Prosthetic Rehabilitation of Postsurgical Nasomaxillary Hypoplasia for a Patient Following Reconstructive Surgery: A Clinical Report. J Prosthodont 2011; 20 : 224–227.

40. **Verran J, Maryan C J.** Retention of Candida albicans on acrylic resin and silicone of different topography. J Prosthet Dent 1997; 77: 535-9.

41. **Vieira A P C, Senna P M, Silva W J S, Del Bel Cury A A**. Long-term efficacy of denture cleansers in preventing Candida spp. Biofilm recolonization on liner surface. Braz Oral Res 2010; 24; 3:342-8.

42. **Vural C, Ozdemir G, Kurtulmus H, kumbuloglu O and Mutlu Özcan.** Comparative effects of two different artificial body fluids on Candida albicans adhesion to soft lining materials. Dent Mat Journal 2010; 29: 206–212.

43. **Waltimo T,Vallittu P, Haapasalo M.** Adherence of Candida species to Newly polymerized and water stored. Int J prosthodont 2001; 14: 457 – 460.

44. **Ye Jin, samaranayake L P, Samaranayake Y, Hak Kong Yip**. Biofilm formation of Candida albicans is variably affected by saliva and dietary sugars. Archives of Oral Biology 2004: 49: 789-798.

9. Annexure.

ANNEXURE I

CANDIDAL ADHERENCES (No .OF COLONIES) ON FLEXIBLE DENTURE BASE RESIN SAMPLES AS VIEWED UNDER OPTICAL MICROSCOPE

S. No	VALPLAST (AFTER 16 HRS)		VALPLAST (AFTER 32 HRS)	
	CLEANED	UNCLEANED	CLEANED	UNCLEANED
1	9	15	5	105
2	11	18	7	135
3	10	16	8	109
4	10	27	7	103
5	9	26	6	115
6	8	15	7	125
7	10	18	4	120
8	11	12	8	120
9	11	15	4	105
10	10	18	5	110
11	10	35	9	123
12	11	23	6	132
13	12	20	8	110
14	9	21	6	130
15	8	28	7	115

ANNEXURE II

CANDIDAL ADHERENCES (No .OF COLONIES) ON CONVENTIONAL POLYMETHYL METHACRYLATE RESIN SAMPLES AS VIEWED UNDER OPTICAL MICROSCOPE

S .No	CONVENTIONAL PMMA (AFTER 32 HRS)		CONVENTIONAL PMMA (AFTER 32 HRS)	
	CLEANED	UNCLEANED	CLEANED	UNCLEANED
1	0	4	0	5
2	0	3	0	8
3	0	2	1	8
4	1	2	0	4
5	0	4	0	12
6	0	2	0	4
7	0	3	0	5
8	1	2	0	8
9	0	3	0	10
10	0	2	1	5
11	0	2	0	5
12	1	1	0	6
13	0	2	0	8
14	0	2	0	6
15	0	3	0	7